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1. Your reference — 100759

2. Patent application number *(The Patent Office will fill in this part)* — 0216700.5 — 18 JUL 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

AstraZeneca AB
S-151 85 Sodertalje
Sweden

Patents ADP number *(if you know it)* 78224480 03

If the applicant is a corporate body, give the country/state of its incorporation — Sweden

4. Title of the invention

PROCESS

5. Name of your agent *(if you have one)*

Michael Andrew Nelson

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

AstraZeneca UK Limited
Global Intellectual Property
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG

Patents ADP number *(if you know it)* 78224710 02

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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Claim(s)	1 ✓
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Signature [signature] Date

Authorised Signatory 17/07/2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Jennifer C Bennett - 01625 230148

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PROCESS

The present invention relates to a process for the preparation of a dispersion of crystalline nano-particles in an aqueous medium, more particularly to a process for the preparation of a dispersion of crystalline nano-particles comprising a substantially water-insoluble pharmacologically active compound in an aqueous medium.

Dispersions of a solid material in a liquid medium are required for a number of different applications including paints, inks, dispersions of pesticides and other agrochemicals, dispersions of biocides and dispersions of pharmacologically active compounds.

In the pharmaceutical field many pharmacologically active compounds have very low aqueous solubility which can result in low bioavailability when such compounds are administered to a patient. Generally, the bioavailability of such compounds is improved by reducing the particle size of the compound, particularly to a sub-micron size , because this improves dissolution rate and hence absorption of the compound.

The formulation of a pharmacologically active compound as an aqueous suspension, particularly a suspension with a sub-micron particle size, enables the compound to be administered intravenously thereby providing an alternative route of administration which may increase bioavailability compared to oral administration.

Formation of suspensions of crystalline nano-particles (nano-crystals) through direct precipitation is known in the art to be problematic. The rapid precipitation necessary to achieve small particle size usually results initially in a suspension of amorphous material. Although this will often crystallise over time, slow crystal growth will tend to result in a particle size of >1 micron.

Formation of crystalline dispersions obtained by precipitation are known in the art to be influenced by agitation of the solutions. Various methods of agitation are known in the art (see for example, WO 01/92293), for example mechanical mixing, vibration, micro-wave treatment and sonication.

In a novel method of obtaining crystalline nano-particles, Kasai et al (Jpn. J. Appl. Phys., **31**, L1132 (1992)) precipitated particles by dropwise addition of an ethanol solution of an organic compound (typically 50 µl with a concentration of 30 mM) into 10 ml of vigorously stirred water giving a total concentration of approximately 0.15 mM. Stirring was then continued for a few minutes and the particle size obtained was about 300 nm. They found

that the particle size could be reduced by precipitation at still lower concentrations. By the same procedure Kasai et al (Bull Chem Soc Jpn, 71, 2597 (1998)) formed aqueous suspensions of nano-crystals of perylene at concentrations between 2.5 and 20 μM. However, such low concentrations generally require the sample to be concentrated e.g. by ultrafiltration, before use. Furthermore, if the total initial concentration of organic compounds is increased then the size of particles obtained by such methods as those mentioned above is >1μm. (see e.g. F.Ruch, E. Matijevic, Journal of Colloid and Interface Science, **229**, 207 (2000)).

An alternative approach to direct precipitation is to reduce the particle size of the material prior to suspension, for example by milling, however this can be disadvantageous as it may be difficult to achieve a sufficiently uniform crystal size. Suspension of material with a non-uniform crystal size will tend to encourage crystal growth. The growth of particles in the dispersion may result in instability of the dispersion during storage and the precipitation of particles from the dispersion.

It is particularly important that the particle size in a dispersion of a pharmacologically active compound remains constant because a change in particle size is likely to affect the bioavailability and hence the efficacy of the compound. Furthermore, if the dispersion is required for intravenous administration, growth of the particles in the dispersion may render the dispersion unsuitable for this purpose, possibly leading to adverse or dangerous side effects.

We have surprisingly found that dispersions of nano-crystals in an aqueous medium can be prepared using a direct precipitation process, wherein the crystallisation is induced by application of ultrasound.

According to a first aspect of the present invention there is provided a process for the preparation of dispersion of nano-crystalline particles in an aqueous medium comprising:

combining with rapid mixing:

a) a first solution comprising a substantially water-insoluble substance in a water-miscible organic solvent with

b) an aqueous phase comprising water and optionally a stabiliser,

and thereafter sonicating the resulting mixture for a sufficient period to form nano-crystalline solid particles of the substantially water-insoluble substance; and optionally removing the water-miscible organic solvent.

In this specification, by crystalline nano-particles or nano-crystals or nano-crystalline particles, we mean crystalline particles with a particle size of less than one micron.

The crystals in the dispersion preferably have a mean particle size of less than 1μm and more preferably less than 500nm. It is especially preferred that the crystals in the dispersion have a mean particle size of from 10 to 500nm, more especially from 50 to 250nm and still more especially from 100 to 200nm. The mean particle size (hydrodynamic diameter) of the crystals in the dispersion may be measured using conventional techniques, for example by dynamic light scattering using a BeckmanCoulter N4 plus apparatus. The crystals may also suitably be examined and their size and shape determined using cryo Transmission Electron Microscopy, for example a Zeiss EM 902.

The substantially water-insoluble substance in the first solution is preferably a substantially water-insoluble organic substance. By substantially insoluble is meant a substance that has a solubility in water at 25°C of less than 0.5 mg/ml, preferably less than 0.1mg/ml and especially less than 0.05mg/ml

The solubility of the substance in water may be measured using a conventional technique. For example, a saturated solution of the substance is prepared by adding an excess amount of the substance to water at 25°C and allowing the solution to equilibrate for 48 hours. Excess solids are removed by centrifugation or filtration and the concentration of the substance in water is determined by a suitable analytical technique such as HPLC.

The process according to the present invention may be used to prepare aqueous dispersions of nano-crystalline particles of a wide range of substantially water-insoluble substances. Suitable substances are those which are known to be able to crystallise in at least one solvent-nonsolvent system. Suitable substances include but are not limited to pigments, pesticides, herbicides, fungicides, industrial biocides, cosmetics and pharmacologically active compounds.

A further aspect of the invention comprises nano-crystalline particles of a substance made by the process of the current invention.

In a preferred embodiment the substantially water-insoluble substance is a substantially water-insoluble pharmacologically active compound. Numerous classes of pharmacologically active compounds are suitable for use in the present invention including but not limited to, substantially water-insoluble anti-cancer agents, steroids, preferably glucocorticosteroids (especially anti-inflammatory glucocorticosteroids, for example budesonide) antihypertensive agents (for example felodipine or prazosin), beta-blockers (for example pindolol or propranolol), hypolipidaemic agents, aniticoagulants, antithrombotics, antifungal agents (for example griseofulvin), antiviral agents, antibiotics, antibacterial agents

(for example ciprofloxacin), antipsychotic agents, antidepressants, sedatives, anaesthetics, anti-inflammatory agents (for example ketoprofen), antihistamines, hormones (for example testosterone), immunomodifiers, or contraceptive agents.

Sonication

By sonication, we mean application of ultrasound to the mixture resulting from the combination of the first solution and the aqueous phase.

According to the process of the present invention, combination of the first solution and the aqueous phase with rapid mixing results in an initial precipitate of amorphous particles of the substantially water-insoluble compound, wherein the amorphous particles have a particle size <1μm.

After combination is complete, the mixture is sonicated until crystallisation of the amorphous precipitate occurs. A sufficient period for sonicating the mixture after combination is therefore a period sufficient for complete conversion of amorphous particles into crystalline particles. A suitable sufficient period is for example 10 – 200 minutes, preferably 10-120 minutes and especially 20-100 minutes. It will be appreciated that the time required may depend upon a number of factors, for example the nature of the sparingly-water soluble compound, the ultrasound frequency, the volume of the solutions used and energy output of the sonication equipment.

Conveniently on a laboratory scale, sonication equipment with an ultrasonication frequency of 35 kHz and a power output of 285W may be used, for example an Elma Transsonic Bath T460/H. On a non-laboratory scale a sonoreactor, for example from AEA Technology could be used.

Water-Miscible Organic Solvent

The water-miscible organic solvent in the first phase is preferably miscible with water in all proportions. The water-miscible organic solvent should also be a solvent for the substantially water-insoluble substance. The water-miscible organic solvent is selected such that the substantially water-insoluble substance has a sufficient solubility in the water miscible organic solvent to enable a precipitate of the substantially water-insoluble substance to form when the first solution is combined with the aqueous phase. Suitably, the substantially water-insoluble substance has a solubility of 10mg/l or more in the water-miscible organic solvent.

Generally it is preferred that the concentration of the substantially water-insoluble substance in the water-miscible organic solvent is as high as possible to aid efficient precipitation. The upper concentration of the substantially water-insoluble substance in the water-miscible organic solvent is determined by the solubility of the substance in the solvent. However, we have found that a wide range of concentrations may be used in the present process. Typically, a concentration of substantially water-insoluble substance of 1% by weight or more in the organic solvent is sufficient.

The substantially water-insoluble substance should be fully dissolved in the water-miscible organic solvent. The presence of particles of the substantially water-insoluble substance may result in poor control of the particle size distribution in the dispersion.

If required the solubility of the substantially water-insoluble substance in the water-miscible organic solvent can be increased by heating a mixture of the substantially water-insoluble substance and water-miscible organic solvent to provide a solution. The solution is then maintained at elevated temperature until it is combined with the aqueous phase in the process.

As will be understood, the selection of water-miscible organic solvent will be dependent upon the nature of the substantially water-insoluble substance. When the substantially water-insoluble substance is an organic compound the water-miscible organic solvent should have a sufficiently low dielectric constant to be able to dissolve the substantially water-insoluble substance. Suitable water-miscible solvents for dissolving a substantially water-insoluble organic substance include, a water-miscible alcohol, for example methanol, ethanol, n-propyl alcohol, isopropyl alcohol, tert-butyl alcohol, ethylene glycol or propylene glycol; dimethylsulfoxide; dimethylformamide; a water-miscible ether, for example tetrahydrofuran; a water-miscible nitrile, for example acetonitrile; a water-miscible ketone, for example acetone or methyl ethyl ketone; dimethylacetamide or a mixture of two or more of the above mentioned water-miscible organic solvents.

Precipitation

In the present process the first solution and the aqueous phase may conveniently be combined by adding the first solution to the aqueous phase with rapid mixing. Conveniently, rapid mixing of the two solutions during combination may be achieved by sonication during the combination. Alternatively other agitation methods known in the art may be used,

provided that the rate of agitation is sufficiently high to result in amorphous particles with a <1μm particle size.

Some particles will precipitate and form a uniform dispersion without the need for a stabiliser in the aqueous phase. However, we have found that many particles tend to aggregate upon precipitation unless a stabiliser is present in the aqueous phase.

Stabilisers suitable for the prevention of particle aggregation in dispersions are well known to those skilled in the art. Suitable stabilisers include dispersants and surfactants which may be anionic, cationic or non-ionic. Suitable dispersants include, a polymeric dispersant, for example a polyvinylpyrrolidone, a polyvinylalcohol or a cellulose derivative, for example hydroxypropylmethyl cellulose, hydroxy ethyl cellulose, ethylhydroxyethyl cellulose or carboxymethyl cellulose. Suitable anionic surfactants include salts of alkyl and aryl sulphonic acids for example, sodium dodecyl sulphate. Suitable cationic surfactants include quaternary ammonium compounds and fatty amines. Suitable non-ionic surfactants include, monoesters of sorbitan which may or may not contain a polyoxyethylene residue, ethers formed between fatty alcohols and polyoxyethylene glycols, polyoxyethylene-polypropylene glycols, an ethoxylated castor oil (for example Cremophor EL), ethoxylated hydrogenated castor oil, ethoxylated 12OH-stearic acid (for example Solutol HS15). The aqueous phase may contain a single stabiliser or a mixture of two or more stabilisers. In a preferred embodiment the aqueous phase contains a polymeric dispersant and an anionic surfactant, for example a polyvinylpyrrolidone and sodium dodecyl sulphate. When the substantially water-insoluble material is a pharmacologically active compound it is preferred that the stabiliser is a pharmaceutically acceptable material.

Generally the aqueous phase will contain from 0.01 to 1% by weight, preferably from 0.05 to 0.5% by weight and especially from 0.1 to 0.2% by weight of stabiliser.

Optionally, additional stabiliser may be added to the dispersion after precipitation of the particles into the aqueous phase to provide additional inhibition of particle aggregation in the dispersion.

The combination of the first solution and aqueous phase in the process according to the present invention results in very fast, substantially instantaneous precipitation of particles of the substantially water-insoluble material to give particles of the desired size with a narrow particle size distribution. Prolonged sonication of this suspension results in formation of nano-crystals of the desired size with a similarly narrow particle size distribution.

crystals but instead use the dispersion as formed, for example because isolation of crystals of a particular substance results in agglomeration.

In another embodiment of the present invention the process is performed under sterile conditions, thereby providing a sterile dispersion directly which can be administered to a warm blooded mammal as described above without the need for additional purification or sterilisation steps. Alternatively, the dispersion may be sterile filtered following crystallisation and optional removal of the water-miscible organic solvent to leave a sterile suspension.

According to a further aspect of the present invention there is provided an aqueous dispersion comprising a continuous aqueous phase in which is dispersed nano-crystalline particles of a substantially water-insoluble substance obtainable by the process according to the present invention.

Preferably the substantially water-insoluble substance is a substantially water-insoluble pharmacologically active material as described above.

When the substance is a substantially water-insoluble pharmacologically active material, the dispersions according to the present invention may be administered to a warm blooded mammal (especially a human), for example by oral or parenteral (e.g. intravenous) administration. In an alternative embodiment the dispersion may be used as a granulation liquid in a wet granulation process to prepare granules comprising the substantially water-insoluble pharmacologically active material and one or more excipients. The resulting granules may then be used directly, for example by filling into capsules to provide a unit dosage containing the granules. Alternatively the granules may be optionally mixed with further excipients, disintegrants, binders, lubricants etc. and compressed into a tablet suitable for oral administration. If required the tablet may be coated to provide control over the release properties of the tablet or to protect it against degradation, for example through exposure to light. Wet granulation techniques and excipients suitable for use in tablet formulations are well known in the art.

Process

In the following Examples, sonication was carried out using an Elma Transsonic Bath T460/H with a volume of 2.75 liter, a power consumption of 285 W and an ultrasonication frequency of 35 kHz.

Images shown in the Examples section are taken with cryo-TEM (cryo Transmission Electron Microscopy) using undiluted nanoparticle suspensions at 25 °C in CEVS (Controlled Environment Vitrification system). The samples were applied as a thin film on a metal plate coated with a porous polymer film, vitrified in liquid ethane at -170 °C and studied at the boiling temperature of nitrogen in a Zeiss EM 902 (accelerator voltage 80 kV).
The mean particle hydrodynamic diameters referred are intensity-weighted numbers obtained from dynamic light scattering measurements.

Examples

The current invention will be illustrated but not limited by the following examples.

Example 1 - Felodipine

A solution of 100 mM Felodipine in dimethylacetamide (DMA) was prepared. 0.010 ml of this solution was added rapidly to 0.990 ml of an aqueous solution containing 0.2%w/w polyvinylpyrrolidone (PVP) and 0.25 mM sodium dodecyl sulfate (SDS) under sonication. The sonication was continued for 30 minutes. The resulting particles were crystalline with a mean hydrodynamic diameter of 165 nm (no change in particle size was observed over 2 hours). Cryo-TEM images of the particles are shown in Figure 1a.

Comparative Example 1

The process of Example 1 was repeated but sonication was discontinued directly after mixing the two solutions. The process produced amorphous particles with a mean particle hydrodynamic diameter of approximately 170nm. The particle size increased due to Ostwald ripening over a period of 1 hour from 170 to 250 nm, and after 2 hours the size was 370 nm. A cryo-TEM image of the particles taken approximately 20 minutes after mixing are shown in Figure 1b.

Example 2 – Candesartan cilexitil

A solution of Candesartan cilexitil (100 mM) in dimethylacetamide (DMA) was prepared. 0.010 ml of this solution was added rapidly to 0.990 ml of an aqueous solution containing 0.2%w/w polyvinylpyrrolidone (PVP) and 0.25 mM sodium dodecyl sulfate (SDS) under sonication. The sonication was continued for 75 minutes. The resulting particles were crystalline with a mean hydrodynamic diameter of 170 nm.

In a comparative experiment Example 2 was repeated except sonication was discontinued directly after mixing the two solutions, amorphous particles were obtained with a mean hydrodynamic diameter of 70 nm.

Brief Description of the Figures

Figure 1a shows cryo-TEM images of Felodipine nano-crystals.

Figure 1b shows cryo-TEM images of Felodipine amorphous particles.

CLAIMS

1. A process for the preparation of a dispersion of nano-crystalline particles in an aqueous medium comprising:

combining with rapid mixing:

(a) a first solution comprising a substantially water-insoluble substance in a water-miscible organic solvent with

(b) an aqueous phase comprising water and optionally a stabiliser,

and thereafter sonicating the resulting mixture for a sufficient period to form nano-crystalline solid particles of the substantially water-insoluble substance; and optionally removing the water-miscible organic solvent.

2. A process according to claim 1 wherein the substantially water-insoluble substance is a substantially water-insoluble pharmacologically active compound.

3. A process according to any one of the preceding claims wherein the aqueous phase contains a stabiliser.

4. A process according to claim 3 wherein the stabiliser comprises a polymeric dispersant and an anionic surfactant.

5. A process according to claim 4 wherein the polymeric dispersant is polyvinylpyrrolidone and the anionic surfactant is sodium dodecyl sulphate.

Figures

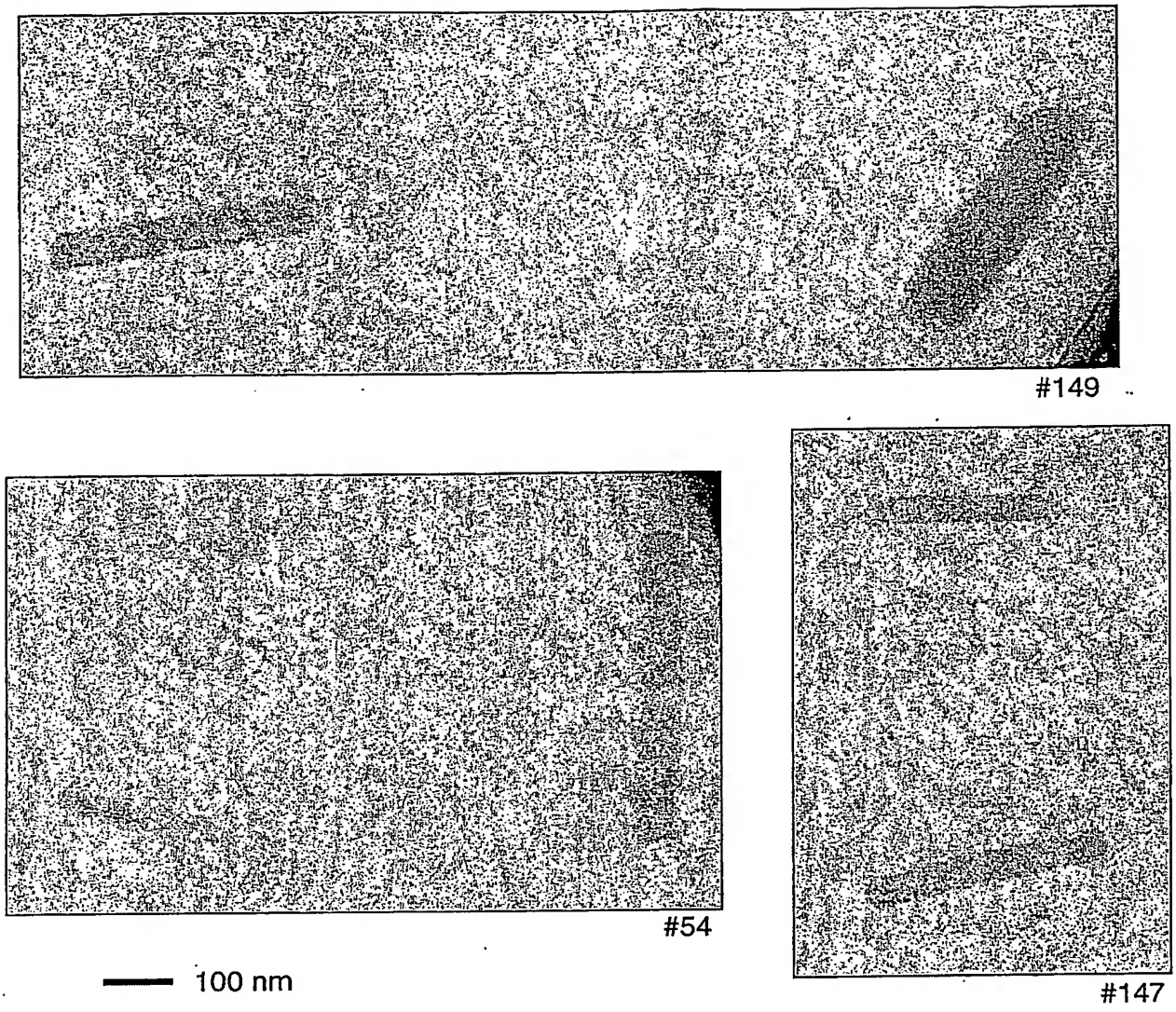

Figure 1a. Cryo-TEM images of crystalline nanoparticles of Felodipine.

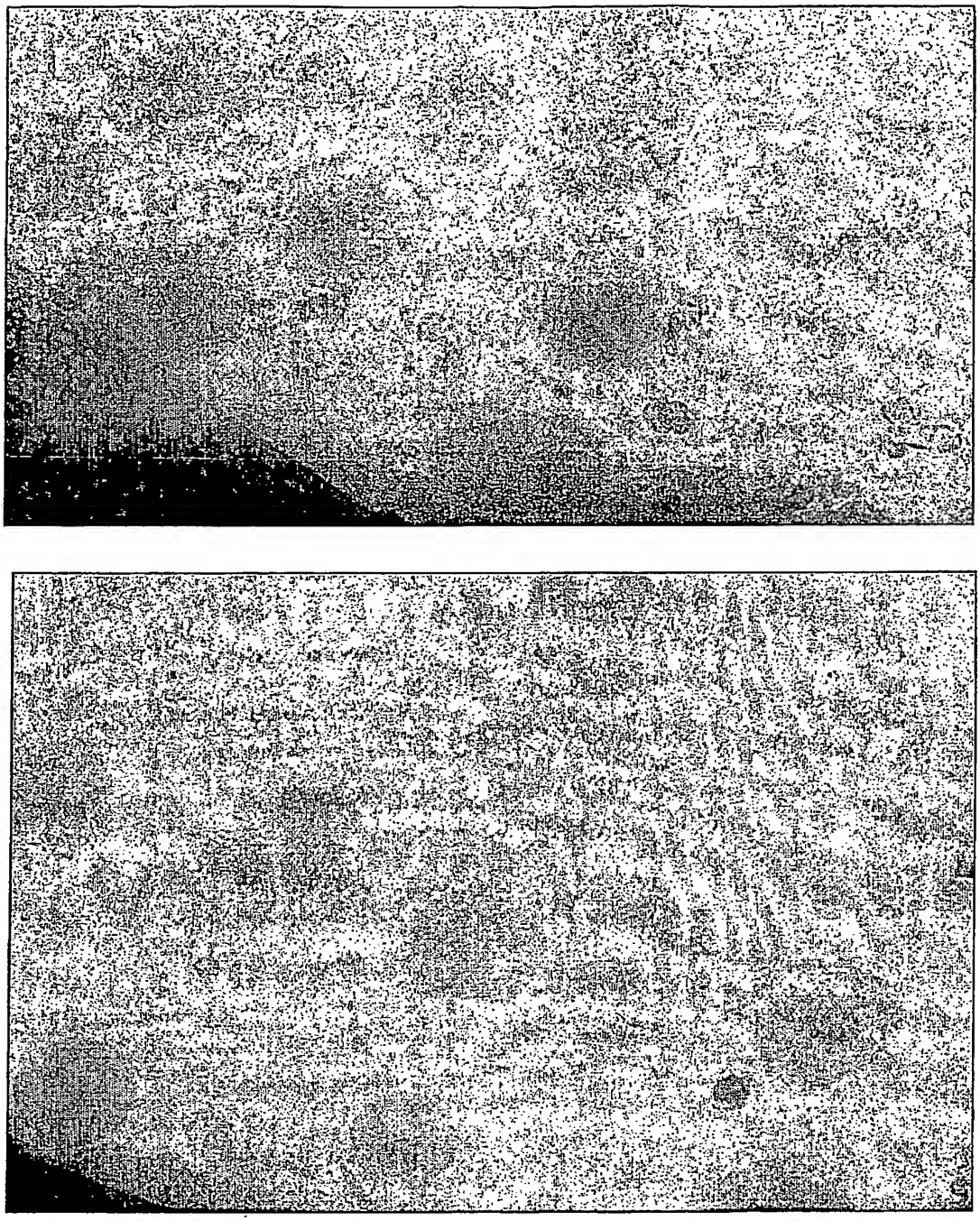

Figure 1b. Cryo-TEM images of amorphous nanoparticles of Felodipine.